Validation of an LC-MS/MS Method for the Determination of Dronedarone (Mutlaq’t) in Human Plasma

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OBJECTIVES:
- Validation of a liquid-liquid extraction of dronedarone from plasma samples to provide clean samples compatible with LC-MS/MS.
- Validation of a sensitive and selective LC-MS/MS method.
- Specificity of a deuterated internal standard to track dronedarone extraction and injection onto the LC-MS/MS for enhanced precision and accuracy (see Figure 1).
- Establish intra- and interday precision and accuracy for the method according to the guidelines.
- Establish stability of dronedarone in solution samples, biological matrix, and injection solvent for the time points and temperature required for analysis of clinical samples.
- Obtain consistent extraction recovery and consistent peak responses from the LC/MS/MS instrument for multiple lots of plasma, including hemolyzed and lipemic lots.

METHODOLOGY:
- Internal standard, 6-dronedarone (BSU12 potency, 98% purity pump.
- Dronedarone and 6-dronedarone stock solutions were 105 μg/mL, methanol, stored at -20 °C.
- Plasma free of significant interference at the retention time and mass transition of dronedarone and 6-dronedarone [IS] was used for control matrix to prepare calibration standard and quality control (QC) samples.
- Human plasma (EDTA) containing the analyte and internal standard was extracted using a liquid-liquid extraction procedure.
- 0.500 mL of each plasma sample was added in 2 mL 96 well plate.
- Calibration standards contained 1.00, 2.00, 5.00, 10.0, 25.0, 50.0, 100, 200, or 250 ng/mL added for the spiking plasma.
- Quality control (QC) samples contained 1.00 (LLOQ), 50.0, 175, or 500 μM (ULOQ) ng/mL, dronedarone in human plasma. Dilution QC samples were diluted 10-fold with blank control human plasma at the time of analysis.
- Working internal standard (0.05 ng/mL, 6-dronedarone in acetonitrile, 0.050 mL) was added into each sample. A spike of actonitride (ACN) was substituted for double blank samples.

RESULTS:
- Dronedarone response was linear from 1 – 250 ng/mL in K EDTA human plasma. Precision and accuracy of 6-dronedarone concentrations back-calculated from the calibration curve are summarized in Table 1.
- Precision and accuracy of quality control (QC) samples were within acceptance criteria. A summary of statistics for intra- and inter-batch QC is depicted in Table 2.
- No significant interference at the retention time and mass transition of dronedarone or 6-dronedarone was observed from endogenous compounds in any of the 10 human plasma samples (EDTA) extracted.
- No significant matrix effect was observed in any of the 10 human plasma samples (EDTA) that were fortified with 6-dronedarone at the concentration of the LLOQ or the LLOQ (175 ng/mL). See Table 3.
- Accurate quantification and stability of the method was demonstrated in the presence of fentanyl (20,000 ng/mL).

CONCLUSIONS:
- Dronedarone in fresh whole blood and fresh human plasma that is not kept under low-temperature conditions is apparently prone to degrada-
- Dronedarone was demonstrated to require ice-cold temperatures while in fresh human plasma to remain stable.

Method Not Implicated

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